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Association of Semen Attributes and Seminal Plasma Proteins of Buffalo Bulls

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ABSTRACT

This study was conducted to explicate the association of semen attributes with seminal plasma proteins of buffalo bulls. Total 108 ejaculates were collected from six sexually mature adult Bhadawari buffalo bulls aged 2-4 years in three seasons (rainy, winter and summer) of a year by using artificial vagina. Immediately after collection, semen samples were divided into two aliquots. One aliquots of neat semen were evaluated for ejaculate volume (EV), sperm concentration (SC), mass motility (MM), progressive motility (PM), percent live-dead (LD) count, percent Hypo-osmotic swelling test (HOST), and percent acrosomal integrity (AI). The other semen aliquots were centrifuged for harvesting the seminal plasma. SDS-PAGE was performed for separation of seminal plasma proteins and gel images were analysed to determine molecular weights, IOD of protein bands and relative protein fractions (protein %) using the Gel doc system. The correlation results revealed positive correlation of SC with 70 and 72 kDa proteins while negative correlation with 86 kDa protein. The PM showed positive correlation with 24.5, 70 and 72 kDa proteins and negative correlation with 84 and 86 kDa proteins and AI showed positive correlation with 18.5, 24.5, 44.5, 70, and 72 kDa and negative correlation with 20 and 84 kDa proteins. The results of correlation among seminal plasma proteins showed positive correlation of 24.5 kDa with 35, 44.5, 70 and 72 kDa and negative correlation with 86 kDa proteins. The 70 and 72 kDa proteins showed positive correlation with 18.5 and 24.5 kDa and negative correlation with 20, 84 and 86 kDa proteins. The 84 kDa proteins showed negative association with 24.5, 70 and 72 kDa proteins while 86 kDa proteins showed negative association with 24.5, 35, 36.5, 70 and 72 kDa proteins. In conclusion, though significant correlations among seminal plasma proteins and semen characteristics were detected, yet it is noteworthy that correlation does not mean cause. Therefore, more refined studies that allow higher-resolution separation of seminal plasma proteins and more detailed characterization of those proteins, as well as investigation of their physiological role, will further advance knowledge in this area.

Keywords: buffalo, seminal plasma proteins, semen characteristics, correlation

In recent decade there has been important progress in the identification and characterization of many seminal plasma proteins of animals. These proteins are components of seminal glands secreted into seminal plasma and are reported to play important role during sperm capacitation and fertilization (Rodríguez-Martínez *et al.,* 1998), maintenance of plasma membrane stability (Desnoyers and Manjunath, 1992), motility (Henricks *et al.,* 1998; Sánchez-Luengo *et al.,* 2004), capacitation (Therien *et al.,* 1998), viability (Brandon *et al.,* 1999), sperm-egg interaction and fertilization (Yanagimachi, 1994). In addition, seasonal variations have also been reported on semen attributes, biochemistry and seminal plasma proteins of buffalo semen (Farooq *et al.,* 2013;

Sharma *et al.,* 2014; Pandey *et al.,* 2014a; Pandey *et al.,* 2014b). Keeping in mind the significant role of seminal plasma proteins on sperm functions, it is hypothesize that absence, presence, under- or over-expression of specific proteins could alter sperm functions, jeopardizing semen quality and fertilising abilities. Thus present study has been designed to elucidate the association between individual seminal plasma proteins and semen characteristics of buffalo bull.

Table 1. Seasonal variations in Environment Temperature (ºC) and Relative Humidity (%).

Parameters	Rainy season	Winter season	Summer season
Environment Temperature (ºC)	29.47±0.07^{a}	12.15±0.63^{b}	35.16±0.13^{c}
Relative Humidity (%)	80.77±0.70^{a}	83.38±0.83^{b}	49.66±1.30^{c}

Means bearing at least one common superscript in one parameter did not differ significantly (P<0.05), otherwise significant at 5% level (P<0.05).

Table 2: Correlation among semen characteristics of Bhadawari buffalo bull semen

Attributes	MM	SC	PM	LD	HOST	AI
MM	1.00	0.42*	0.37*	-0.01	-0.11	-0.16
SC	0.42*	1.00	0.08	0.19	-0.27	0.00
PM	0.37*	-0.08	1.00	0.32*	0.38*	0.45**
LD	-0.01	0.19	0.32*	1.00	0.40*	0.39*
HOST	-0.11	-0.27	0.38*	0.40*	1.00	0.46**
AI	-0.16	0.00	0.45**	0.39*	0.46**	1.00

* $P < 0.05$.
** $P < 0.01$.

MATERIALS AND METHODS

The study was conducted on six sexually mature adult Bhadawari buffalo bulls of 2–4 years of age of Instruction Livestock Farm Complex of the College. The farm is situated at 27° latitude and 78° longitude 160 meter above sea. The average day temperature and humidity of the study period is depicted in Table 1. The selected bulls were maintained in nearly similar nutritional and management conditions throughout the study period and were regularly dewormed and vaccinated against infectious and contagious diseases as per standard schedule. The study period of one year was divided into three seasons; viz. rainy season (July to September), winter season (December to February) and summer season (April to June) as per agro-climatic conditions prevailing in the area of investigation. Total 108 ejaculates were collected from six bulls in morning hours using sterilized artificial vagina (Tomar, 1986). After collection, each semen sample was immediately divided into two aliquots; one aliquot was centrifuged at 5000 rpm for 10 min at 4°C for harvesting seminal plasma and second aliquot was evaluated for semen attributes viz. ejaculate volume (EV), mass motility (MM), sperm concentration (SC), progressively motility (PM) and sperm morphology viz. percent live-dead spermatozoa (LD), percent hypo-osmotic swelling test (HOST) and percent acrosomal integrity (AI) by using standard methods (Sharma *et al.,* 2014). The separated

Table 3. Correlation among seminal plasma proteins and semen characteristics of Bhadawari buffalo bull semen

Proteins (kDa)	EV	MM	SC	PM	LD	HOST	AI
6.5	-0.12	-0.01	-0.00	0.12	-0.09	0.39	-0.20
12.5	-0.30	-0.17	-0.20	0.28	-0.01	0.01	0.32
18.5	0.20	-0.06	0.39	0.41	-0.03	0.21	-0.55*
20	-0.26	-0.06	-0.22	-0.29	-0.11	0.14	-0.59*
24.5	-0.07	0.30	0.20	0.71**	0.140	0.37	0.71**
26.5	-0.18	0.00	-0.15	-0.22	-0.26	0.27	-0.21
28	0.28	0.24	0.31	0.09	-0.07	-0.17	0.18
32	-0.01	-0.25	-0.27	-0.23	0.15	-0.28	-0.29
35	-0.12	0.17	0.19	0.43	0.00	0.37	0.30
36.5	0.25	0.32	0.29	0.02	0.13	-0.26	0.16
38	-0.35	-0.12	0.06	0.16	0.03	0.03	-0.25
44.5	0.15	0.46	0.21	0.48*	0.17	0.16	0.59*
66	-0.55	-0.40	-0.12	0.37	-0.03	0.26	0.16
70	0.38	0.19	0.55*	0.61**	0.17	-0.01	0.56*
72	0.31	0.07	0.51*	0.68**	0.29	0.12	0.65**
84	0.08	-0.02	-0.04	-0.62**	-0.08	-0.32	-0.49*
86	-0.34	-0.29	-0.60**	-0.52*	-0.23	0.16	-0.39
96	-0.50*	-0.20	-0.12	0.12	-0.12	0.13	-0.10

* $P < 0.05$.
** $P < 0.01$.

seminal plasma was subjected to protein extraction by Triprep extraction kit (Fisher Scientific). Proteins were recovered and re-suspended in phosphate buffered saline (PBS) and stored at −20°C until electrophoresis was performed. Before performing SDS PAGE for protein analysis, the samples were thawed and all the samples of each bull were pooled. These pooled samples were centrifuged at 3000 rpm for 1 h at 4°C and total protein was determined by Biuret colorimetric method just before electrophoresis.

Table 4. Correlation among seasonally affected seminal plasma proteins of Bhadawari buffalo bull semen

Proteins (kDa)	24.5	66	70	72	84	86
6.5	-0.14	0.00	-0.22	-0.22	0.26	0.12
12.5	0.32	0.42	0.03	0.04	-0.14	-0.17
18.5	0.43	0.28	0.47*	0.54*	-0.15	-0.33
20	-0.39	-0.33	-0.46*	-0.59*	0.07	0.29
24.5	1.00	0.41	0.48*	0.65**	-0.44*	-0.59*
26.5	-0.20	-0.29	-0.32	-0.41	0.29	0.35
28	0.21	0.00	0.18	0.15	0.30	-0.36
32	-0.44	0.20	-0.42	-0.31	0.44	0.24
35	0.65**	0.30	0.10	0.10	0.02	-0.51*
36.5	0.33	0.27	0.29	0.32	0.13	-0.55*
38	0.12	0.67	0.08	0.25	-0.05	-0.20
44.5	0.66**	-0.18	0.37	0.30	-0.36	-0.38
66	0.41	1.00	0.06	0.25	-0.11	-0.32
70	0.48*	0.06	1.00	-0.90**	-0.64**	-0.65**
72	0.65**	0.25	-0.90**	1.00	-0.60**	-0.62**
84	-0.44	-0.11	-0.64**	-0.60**	1.00	0.26
86	-0.59*	-0.32	-0.65**	-0.62**	0.26	1.00
96	0.00	0.35	-0.07	-0.15	-0.06	-0.01

* $P < 0.05$.
** $P < 0.01$.

SDS-PAGE was performed on 12% polyacrylamide gel for separation and determination of molecular weight of various seminal plasma proteins (Laemmli, 1970). The pooled samples were diluted in ultra-purified water at a concentration of 5 µg/µL of total protein mixed with sample buffer at 1:4 buffer/sample dilution, homogenized and boiled (100°C) for 7 min (Desouza *et al.,* 2007). The total loaded quantity of protein in each well was 60 µg in 15 µL. Each gel was also loaded with a standard broad-range molecular weight markers (Merck, Mumbai, India) for separation on gels. The proteins were electrophoresed following loading the samples in wells at constant voltage of 60 V at room temperature for 30 minutes through the stacking gel and at voltage 80V through the separating gel till tracking dye front reached close to the bottom of gel slab. At the end of electrophoresis the gel were removed from gel electrophoresis unit and stained for 2 hour in staining solution of 0.1% Coomassie Brilliant blue G solution. After staining, destaining is achieved by diffusion against three changes of destaining solution (water: methanol: acetic acid) at room temperature and finally the gel was stored in 7% acetic acid for gel documentation analysis. Gel images were digitized (Gel-doc. Model-Alpha imager TM1220, Alpha Innotech Corporation, USA) and molecular weight (MW) and integrated optical density (IOD) were determined for calculating the % protein fraction for each band of a gel.

Pearson correlations coefficient between values of semen characteristics and seminal plasma proteins was calculated to investigate the association among indices using SPSS software for Windows (version 16.0). The data are presented as the mean ± SE. A p value < 0.05 was considered to be statistically significant and p value <0.01 considered highly significant.

RESULTS AND DISCUSSION

The results of Pearson's correlation among semen attributes are depicted in Table 2. The results showed positive association of MM with SC and PM while SC with MM. The PM demonstrated positive correlation with MM, LD, HOST and AI while LD with PM, HOST and AI. The HOST illustrated positive correlation with PM, LD and AI whereas AI with PM, LD and HOST. The significant correlations observed among PM, LD, HOST and AI were also reported in earlier studies (Lodhi *et al.,* 2008; Mandal *et al.,* 2009; El-Sisy *et al.,* 2010; Farooq *et al.,* 2013; Ray and Ghosh, 2013) in cattle and buffalo bull semen. The present study revealed positive association among PM, LD, HOST and AI. Similar positive association among these attributes was reported by Mandal *et al.* (2009) and El-Sisy *et al.* (2010) in buffaloes corroborated the findings of present study. The positive correlation observed

between MM and PM in present is found to be simulated with results of Shukla *et al.* (2009) and Farooq *et al.* (2013) in cattle and buffalo bull, respectively.

The results of Pearson correlation of protein fraction and seminal attributes presented in Table 3. The EV demonstrated positive correlation with 70 kDa and negative correlation with 38, 66, and 86 kDa proteins. The MM illustrated positive association with 44.5 kDa and negative with 66 kDa proteins. The SC showed significant positive correlation with 18.5, 70 and 72 kDa proteins, while negative correlation with 86 kDa protein. The PM revealed positive correlation with 24.5, 35, 44.5, 66, 70 and 72 kDa proteins and negative correlation with 84 and 86 kDa proteins. The HOST exhibited positive correlation with 24.5 and 35 kDa proteins. The AI demonstrated positive correlation with 24.5, 44.5, 70, and 72 kDa proteins while negative correlation with 18.5, 20 84 and 86 kDa ($P<0.05$) proteins. However LD did not reveal significant correlation with any of the seminal plasma protein. The results obtained for correlation between semen characteristics and seminal plasma proteins were found comparable with reports of some authors (Cardozo *et al.,* 2006; Asadpour *et al.,* 2007; Desouza *et al.,* 2007). Asadpour *et al.* (2007) reported significant correlations between protein fractions of 24.5 kDa with sperm progressive motility while 55 kDa protein fractions with sperm viability of fresh semen, corroborate the findings of present study. However negative correlation also reported between bands of 15.9 kDa with sperm viability and concentration and between the relative protein content of 73.2 kDa with viability (Cardozo *et al.,* 2006).

The results of correlations observed among % protein fractions of seminal protein bands are depicted in Table 4. The 24.5 kDa protein showed positive correlation with 18.5, 35, 44.5, 66, 70 and 72 kDa and negative correlation with 20, 32, 84 and 86 kDa proteins. The 66 kDa protein exhibited positive correlation with 24.5 kDa protein. The 70 kDa protein illustrated positive correlation with 18.5, 24.5 and 44.5 kDa and negative with 20, 32, 72, 84 and 86 kDa proteins. The 72 kDa proteins showed positive correlation with 18.5 and 24.5 kDa and negative correlation with 20, 26.5, 70, 84 and 86 kDa proteins. The 84 kDa proteins revealed positive correlation with 32 kDa and negative association with 24.5, 44.5, 70 and 72 kDa proteins while 86 kDa proteins showed positive association with 26.5, and negative association with 24.5, 35, 44.5, 70 and 72 kDa proteins. Present study showed positive correlation of PM with 44.5 kDa and SC with 72 kDa proteins and almost similar correlation was reported by Yue *et al.* (2009) in ram seminal plasma proteins substantiates the findings. However Desouza *et al.* (2007) reported correlation of sperm motility, as well as other semen characteristics, with IOD of two bands (B4 and B5, 67 and 58.6 kDa) and Amann *et al.* (1987) reported linear correlation between motility of both fresh and frozen-thawed spermatozoa and the relative concentration of two protein bands (19.6 and 15.3 kDa) in stallions. These findings indicate that some proteins may modulate sperm function by providing energy and protection for spermatozoa as a complementary substance. This point was also mentioned by Cardozo *et al.* (2006) in his report.

None of the citation was found revealing association among seminal plasma proteins hence results of correlation among seminal plasma proteins could not be discussed.

CONCLUSION

Characterization of seminal plasma proteins has substantial advantage that they can potentially be used to forecast fertility, and perhaps to increase fertility. Although significant correlations between seminal plasma proteins and semen characteristics were detected, yet it is noteworthy that correlation does not mean cause. This fact could support the hypothesis that seminal plasma proteins act on sperm physiology in different ways. Therefore, these observations should be inferred with caution, pending further studies that directly relate expression of seminal plasma proteins with semen characteristics. In addition, more refined studies that allow higher-resolution separation of seminal plasma proteins and more detailed characterization of those proteins, as well as investigation of their physiological role, will further advance knowledge in this area. Additional studies are necessary to define the types of proteins affecting sperm viability and the mechanisms of their actions.

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